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13. ABSTRACT ***(Maximum 200 Words)***

Our goal is to develop a transgenic mouse model for breast cancer that will allow the *in vivo* activities of tumor-specific T cell clones to be tracked at all stages of tumorigenesis and after various immune interventions. We proposed to "tag" the *neu* oncogene with two defined T cell epitopes so as to confer recognition by available T cell receptor (TCR) transgenic T cells. When expressed as a transgene in mammary epithelium, epitope-tagged *neu* (designated $neu^{OT1/OT2}$) should induce formation of aggressive mammary adenocarcinomas that express the epitope tags and hence are recognizable by adoptively transferred TCR trangenic T cells. To date, we have successfully attached the OT1 and OT2 epitope tags to the *neu* oncogene and shown by in vitro assays that (1) $neu^{OT1/OT2}$ is indeed recognized by OT1- and OT2-specific TCR transgenic T cells, and (2) $neu^{OT1/OT2}$ retains its transforming properties. $neu^{OT1/OT2}$ was then placed downstream of the MMTV promoter, and transgenic mice were generated. Of 13 pups born, one transgene-positive male founder was obtained and is currently being bred to multiple females. Thus, Aim 1 and a portion of Aim 2 have been successfully completed on schedule and without any major difficulties.

14. SUBJECT TERMS
transgenic mouse, tumor immunology, immunotherapy, CD4+ and CD8+ T lymphocytes, adoptive transfer, HER2/neu

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Table of Contents

BC990655 Annual Progress Report

PI: Brad H. Nelson, Ph.D.

Title of Project: Development of a transgenic mouse model for breast cancer that is optimized for the study of T cell based therapeutic strategies

Introduction:

Currently, the development of immune-based therapies for breast cancer is impeded by the lack of an animal model that both mimics spontaneous human disease and is amenable to detailed monitoring of the activities of multiple, defined T cell clones that recognize tumor antigens. In this project, we are creating transgenic mice that are genetically programmed to develop spontaneous mammary tumors expressing defined T cell epitopes. Once such mice are available and tumors have developed, we will adoptively transfer CD4+ and CD8+ T cell clones with specificity for the engineered epitopes. These T cells will then be tracked in vivo and analyzed for functional responses to tumor cells. In future, this system will be used to evaluate the mechanisms and efficacy of immune-based therapeutic and preventative strategies. The specific aims of this proposal are:

(1) To construct a dual epitope-tagged version of the oncogene *neu* that is recognized by two epitope-specific T cell clones while retaining transforming potential;

(2) To assess the ability of epitope-tagged *neu*, upon expression in transgenic mice, to induce the formation of mammary tumors that are recognized by epitope-specific CD4+ and CD8+ T cell clones.

Body:

Aim 1: To construct a dual epitope-tagged version of the oncogene* neu *that is recognized by two epitope-specific T cell clones while retaining transforming potential.

As originally proposed, the OT-1 epitope from chicken ovalbumin was placed at the C-terminus of the activated allele of *neu*. We originally proposed to place the IEα epitope from the MHC Class II gene at the N-terminus of *neu*, however this strategy had to be changed in two ways. First, we decided to use the OT-2 epitope from ovalbumin instead of the IEα epitope due to concerns that there might be some expression of the endogenous IE-α gene in B6 mice, which would complicate the interpretation of T cell responses. Like IE-α, the OT-2 epitope is presented to CD4+ T cells by MHC Class II molecules, is well characterized, and is recognized by a readily available TCR transgenic CD4+ T cell clone. However, the OT-2 epitope has the added advantage of being of non-murine origin and thus absolutely cannot be expressed by normal tissues in these mice. The second change we made was to place the OT-2 epitope at the C terminus of *neu* instead of the N terminus. This was necessitated by our unexpected finding that placement of epitopes near the N terminus of *neu* severely disrupted expression of the molecule. Thus, our final construct has both the OT-1 and OT-2 epitopes in tandem at the C terminus of neu, with the OT-2 epitope occupying the most C-terminal position (Fig. 1). This version of epitope-tagged *neu* was very well expressed in multiple cell lines (data not shown). In addition, we were able to demonstrate by in vitro proliferative assays (described in Aim 1 of the proposal) that both the OT1 and OT2 epitope tags were processed and presented to the appropriate CD8+ and CD4+ TCR transgenic T cells (Fig. 2). Finally, using an *in vitro* transformation assay with NR6 fibroblasts (described in Aim 1 of the proposal), we showed that the OT1 and OT2 epitopes do not interfere with the transforming properties of *neu* (data not shown). Thus, all aspects of Aim 1 have now been successfully completed on schedule, yielding a dual epitope-tagged version of *neu* that is suitable for generating transgenic mice. This corresponds to completion of all items (a-c) listed under Task 1 in the Statement of Work.

Aim 2: To assess the ability of epitope-tagged neu, upon expression in transgenic mice, to induce the formation of mammary tumors that are recognized by epitope-specific CD4+ and CD8+ T cell clones.

As proposed, the dual epitope-tagged version of *neu* described above was inserted into a transgenic expression vector containing the MMTV promoter (obtained from Dr. Tim Lane, UCLA). The insert and vector were thoroughly sequenced to ensure they contained no unintended mutations or alterations. Transgenic C57Bl/6 mice were generated by standard oocyte injection in the Dept. of Comparative Medicine at the University of Washington. Thirteen founder pups were born, and two of these were transgene-positive by PCR of tail-derived genomic DNA. Unfortunately, only one of these two pups (a male) survived to maturity; the other died from causes unrelated to the transgene. A second round of oocyte injections will be performed this June to produce a larger number of founder animals.

The one surviving transgene-positive male is currently being bred to multiple females of both the C57Bl/6 and FVB strains. After that, he will be bred to females harboring a dominant-negative p53 transgene expressed in mammary epithelium. By breeding our transgene onto these three different backgrounds (C57Bl/6, FVB and dominant-negative p53), we hope to obtain a continuum of tumorigenic phenotypes such that we can select the optimal strain combination for future experiments. Thus, we have completed items a-c listed under Task 2 in the Statement of Work, which means we are approximately 2 months ahead of schedule.

Key Research Accomplishments:

The following items have been completed or are underway:

Task 1. To construct a dual epitope-tagged version of the oncogene *neu* that is recognized by two epitope-specific T cell clones while retaining transforming potential (Months 1-12). ***completed**

a. Construct plasmids encoding single ($neu^{IE\alpha}$ and neu^{OVA}) and dual ($neu^{IE\alpha/OVA}$) epitope-tagged versions of *neu*; verify DNA sequence (Months 1-3). ***completed**
b. Evaluate signaling and transforming properties of epitope-tagged and untagged versions of *neu* in cell lines; if problems noted, modify epitopes as needed (Months 4-12). ***completed**
c. In vitro assays to evaluate recognition of IEα and OVA epitopes by CD4+ and CD8+ T cells from TCR-transgenic mice (Months 4-12). ***completed**

Task 2. To assess the ability of epitope-tagged *neu*, upon expression in transgenic mice, to induce the formation of mammary tumors that are recognized by epitope-specific CD4+ and CD8+ T cell clones (Months 13-36). ***underway**

a. Construct MMTV vector with human growth hormone gene at 3' end into which to introduce *neu* transgenes (Months 9-12). ***completed**
b. Insert untagged (*neu*) and dual tagged ($neu^{IE\alpha/OVA}$) cDNAs into MMTV vector (Month 13). ***completed**
c. Provide transgenes to the Dept. of Immunology at the University of Washington and have C57Bl/6 transgenic founder mice generated (Months 14-17). ***underway**
d. Perform PCR on tail DNA of pups (approximately 60 animals); breed transgene-positive founders (10-12 animals) (Months 18-19). ***underway**

Reportable Outcomes:

Funding:

U.S. Department of Defense
B.H. Nelson, P.I.

7/01/01-6/30/04
Direct total costs: $299,631
Direct annual costs: $99,877

Eliciting Autoimmunity to Ovarian Tumors in Mice by Genetic Disruption of T Cell Tolerance Mechanisms

The specific aims of this proposal are:
1. To generate an ovarian tumor cell line that is recognized by antigen-specific CD4+ and CD8+ T cell clones from TCR transgenic mice.
2. To define the mechanisms by which ID8 ovarian tumors evade rejection by tumor-specific CD4+ and CD8+ T cells.
3. To determine whether tumor-specific CD4+ and CD8+ T cells lacking the Cbl-b gene show enhanced functional responses to ovarian tumors.

Conclusions:

The mouse model we are developing should lead to an improved understanding of the immune response to breast cancer and may facilitate the development of novel immune-based therapies or immunopreventive strategies for this disease. Toward this goal, we have now created a dually epitope-tagged version of *neu* that is recognized by the appropriate CD4+ and CD8+ T cells while retaining transforming potential. The generation of transgenic mice expressing this version of *neu* in mammary epithelium is underway, with one transgene-positive male pup obtained to date. In Year 2, we will continue to pursue the goals outlined in Aim 2 of our original proposal. No major changes to the research plan are expected. In summary, this study is ahead of schedule and no major obstacles have been encountered.

References:

None.

Appendices:

See accompanying Figures 1 and 2.

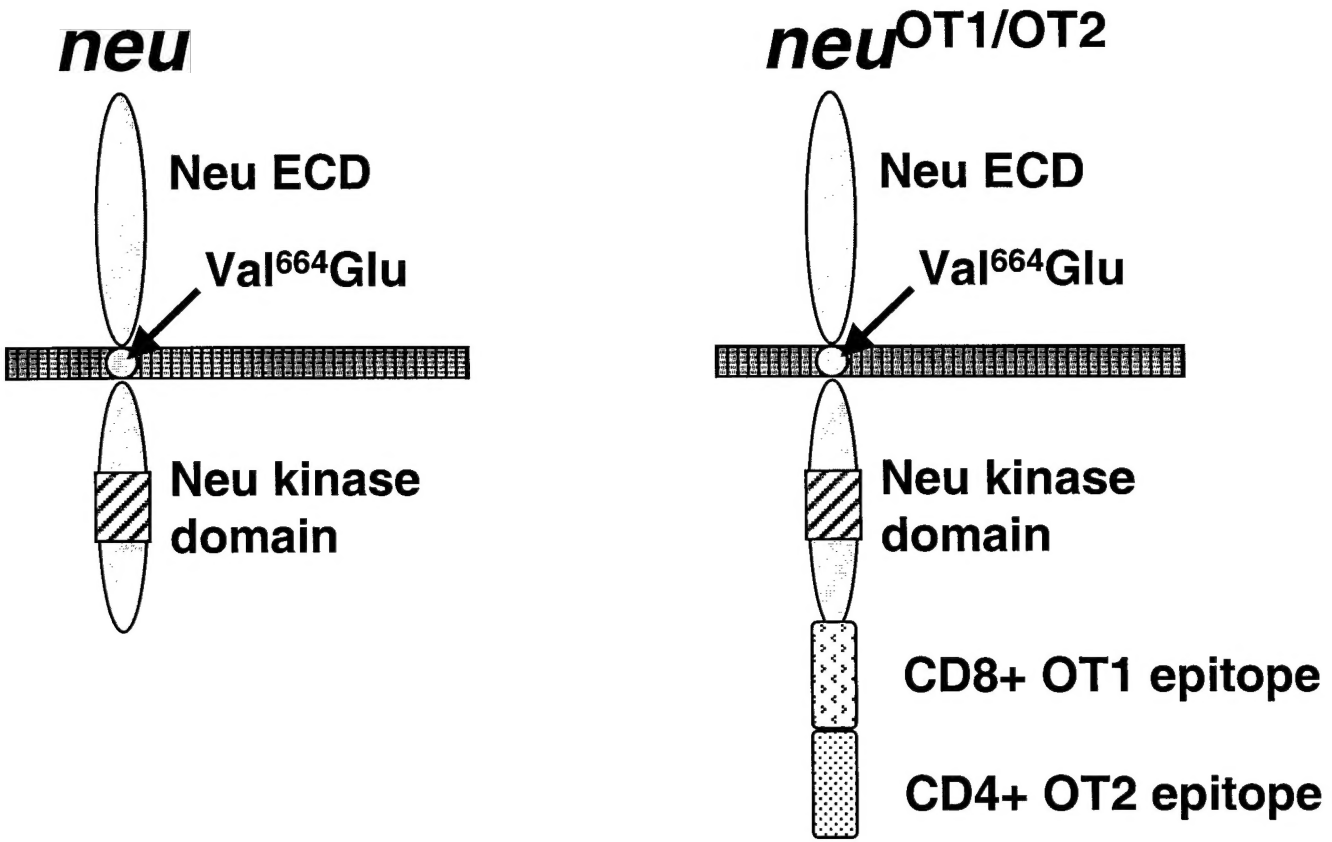

Figure 1. Schematic of the epitope-tagged *neu* oncogene. *Left*, unmodified *neu*. *Right*, epitope tagged *neu*. ECD = extracellular domain.

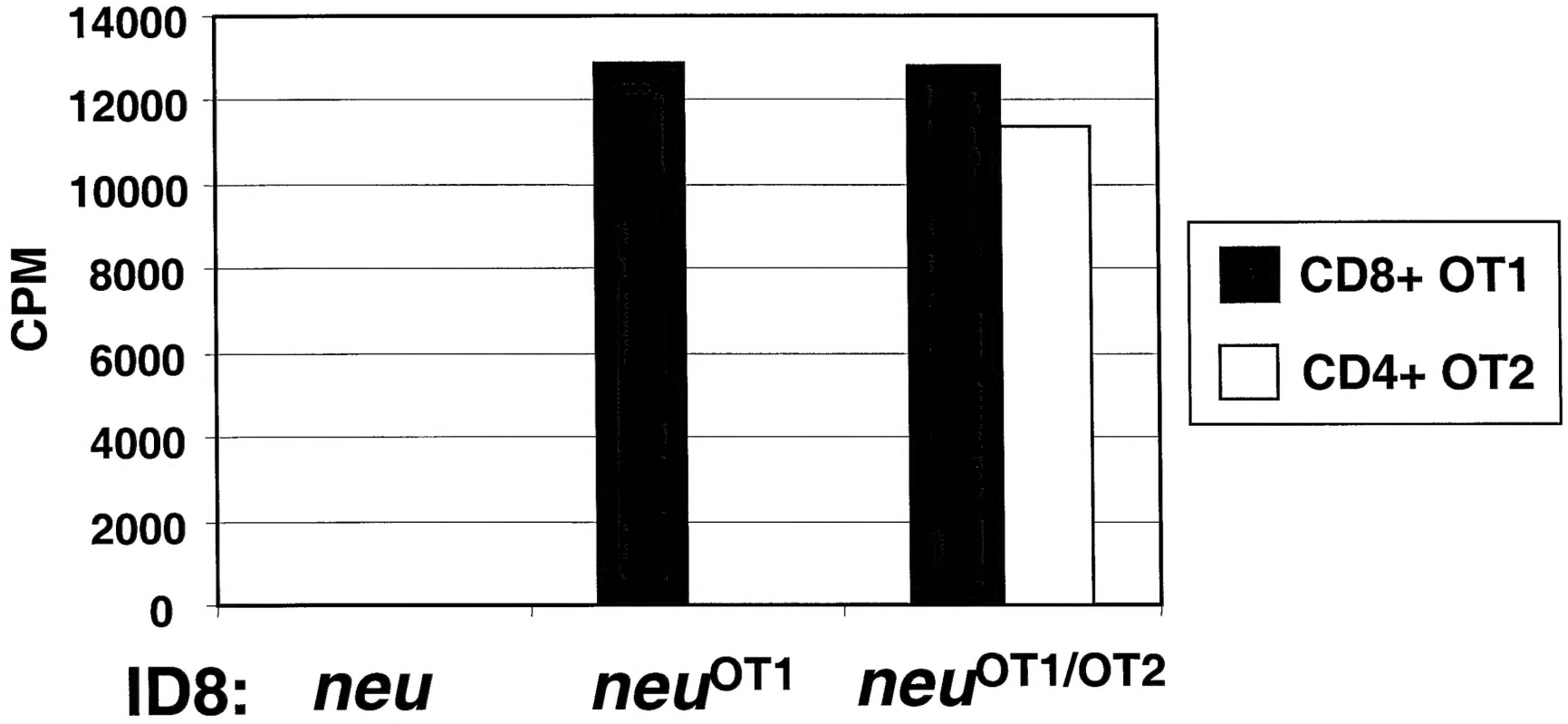

Figure 2. Recognition of epitope-tagged *neu* by CD8+ OT1 and CD4+ OT2 TCR transgenic T cells. Splenocytes from OT1 (black bars) or OT2 (gray bars) TCR transgenic mice were incubated with an epithelial tumor cell line (ID8) that had been transfected to express either untagged *neu*, *neu* bearing a single OT1 epitope tag (*neu*^{OT1}), or dually tagged *neu* (*neu*$^{OT1/OT2}$). Cultures were incubated for 40h, pulsed with tritiated thymidine for 8h, and subjected to liquid scintillation counting.